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研究报告

破骨细胞形成抑制因子TNFR结构区在大肠杆菌中表达、抗体制备及活性测定

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摘要 破骨细胞形成抑制因子(OPG)对骨的重建与再吸收有重要调节作用，其TNFR结构区行使抑制破骨细胞形成与活性的功能。通过PCR将该区基因片段克隆出来，插入表达载体PET-28a质粒多克隆位点，重组质粒转入大肠杆菌BL21中进行表达，表达产物以包涵体形式存在，包涵体经变性复性后，亲合层析获得重组蛋白。纯化的产物作为抗原免疫兔，得到较高特异性的兔源多克隆抗体。利用小鼠降血钙实验检测变性复性后产物的活性，结果表明该重组蛋白有一定的生物活性。

关键词 破骨细胞形成抑制因TNFR结构区；大肠杆菌；抗体；降血钙活性

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Expression and Activity Determination of TNFR Domain of Osteoprotegerin in E.coli and Corresponding Antibody Preparation

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Abstract

Osteoprotegerin (OPG) plays an important role in the regulation of bone resorption and remodeling. The TNFR domain of OPG, which is involved in the inhibition of formation and activity of osteoclasts, was amplified by PCR and inserted into multiple cloning site of PET-28a. The recombinant plasmid was transferred into E.coli BL21 to express recombinant protein. It was found that expressed product existed in the form of inclusion body. The inclusion body was solubilized, renatured and purified by affinity chromatography. Polyclonal antibodies with high specificity were obtained from the serum of rabbit immunized with purified recombinant protein. Mice were used to determine the hypocalcemic effect of the recombinant protein. Results showed that the recombinant protein expressed in E.coli had the proper bioactivity.

Key words osteoprotegerin TNFR domain E.coli antibody hypocalcemic effect

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